Introduction to cryo-electron tomography (cryo-ET)

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What is cryo-ET?





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Single particle cryo-EM vs Cryo-ET



Tiltseries collection schemes



Tiltseries alignment



Tiltseries alignment: Fiducial-based alignment (IMOD)



Gold nanoparticles (10nm diameter)



It is important to optimize the fiducial concentration

Tiltseries alignment: Marker-free alignment (AreTomo)



The missing wedge effect

The deteriorating influence of the missing gap depends on the **tilt range** and **tilt increment**.

2 deg increment

5 deg increment

Bridging cell biology and structure biology



An overview of subtomogram averaging



Particle picking

Direct particle picking



Manual picking, template matching, machine learning

Geometry-based particle picking



https://doi.org/10.1101/2024.04.26.591129

Segmentation-based particle picking



Segmentation



https://doi.org/10.1101/2024.01.05.574336

Segmentation for quantifying organellar ultrastructure



High-resolution subtomogram averaging



Warp/M, EMAN2, Relion 5 ...

Combination of subtomogram averaging and segmentation



Sample thickness limitation



The mean free path is the average distance over which a moving particle (such as an atom, a molecule, or a photon) travels <u>before substantially changing its</u> <u>direction or energy</u> (or, in a specific context, other properties), typically as a result of one or more successive collisions with other particles.

In an **inelastic collision**, the electron transfers energy to the structure and loses its coherence. These electrons damage the specimen and <u>contribute to the</u> <u>background/noise</u>.

The mean free path of 300 keV electrons was determined to be 280 nm for free vesicles in cryogenic conditions (PMID: 8921626, 34873060, 29981485, 32614016)

Bacterial secretion system

T3SS system from Salmonella



PMID: 28283062





Bacterial flagellar motor

Flagellar motor



PMID: 31313986, 32895555

Bacteriology



PMID: 37349588

PMID: 38811725

 NH_4^+

HIV spike

HIV spike with neutralizing antibodies



PMID: 18668044



PMID: 32601441

HIV spike with membrane bound host receptors



HIV capsid



SARS-CoV-2



Thin edges of mammalian cells



PMID: 33139942

Thin the sample <200nm by cryo-focused ion beam (cryo-FIB) milling





Cryo-correlative light and electron microscopy (Cryo-CLEM)



Cryo-CLEM





Dedicated Cryo-CLEM microscope



Cryo-FIB milling



TEM







TEM

Cryo-CLEM



Primary macrophage infected with Salmonella

Cryo-correlative light & electron microscopy (CLEM)



Cryo-Focused Ion Beam (FIB) milling



Cryo-ET



Identifying target of interest from tomogram





PMID: 30281019



PMID: 33606980

Genetically-encoded tag

Mitotic chromosome surface—Ki-67 (HeLa)





Nuclear pore complex—Nup96 (U2OS)





Outlook for Cryo-ET: Interdisciplinary research and "Biology"



Integration of cryo-ET with high-resolution cryo-EM, Albased structure prediction, mass spectrometry, and fluorescence microscopy illustrated by structural studies of intraflagellar transport (IFT)